

Hüseyin Barış Tecimen<sup>1</sup>, Josef Gorres<sup>2</sup> and Ryan Dustin Scott Melnichuk<sup>2</sup>

<sup>(1)</sup> University of Istanbul, Faculty of Forestry, Soil Science and Ecology Dept. Istanbul, Turkey  
<sup>(2)</sup> Plant and Soil Science, University of Vermont, Burlington, VT

## INTRODUCTION

Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide concentrations in the atmosphere all have increased by 42, 140 and 12%, respectively since the 19<sup>th</sup> century (IPCC, 1995). Increased greenhouse gas concentrations are in part due to human activities. However, some mitigation may occur by sequestration into less available compounds in the environment (Denman et al. 2007). Earthworms have recently been implicated in increased greenhouse gas emissions from soils (Lubbers et al. 2013). Earthworms change the microbial community of the soil at least in the rhizosphere (Savin et al. 2004). Concomitant with these changes are aggregate-scale changes in pore structure that increase CO<sub>2</sub> emissions (Gorres et al., 2001). In this study, we were interested in how aggregate scale greenhouse gas emission, nitrate and calcium concentrations, pH were affected by earthworm species and ammonium addition. We hypothesized that additional ammonium may be quickly nitrified by the community left by the earthworms and thus affect nitrate and calcium concentrations.



Figure 1. Pot experiment with soils excavated from spodosol soils under a mixed hardwood/softwood forest.

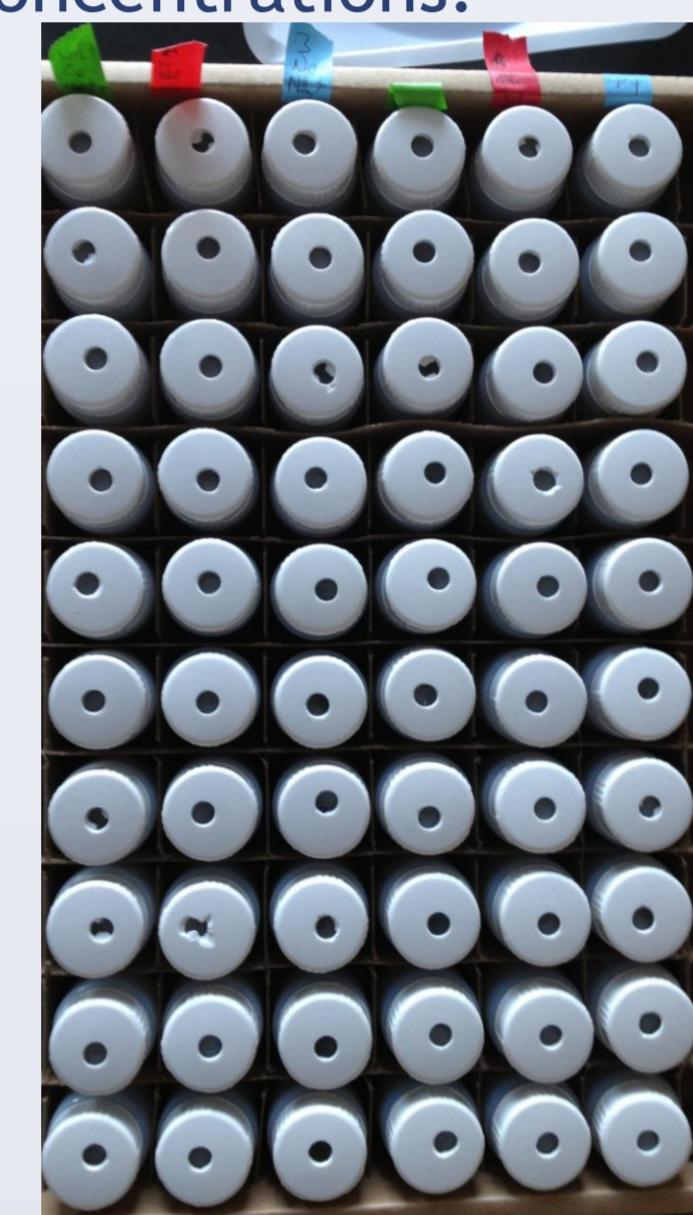


Figure 2. Mesocosm sampling and sealed vials.

All earthworms are exotic to the Northeastern USA and are assumed to change soil structure so that the biodiversity of native understory vegetation is diminished (Hale et al. 2008). There is good evidence that N and C cycles are affected by these invasions. Little is known about base cations and we chose to measure calcium because we found it to be more available at invaded forest sites. We thought that ammonium additions would stimulate nitrification and thus potentially N<sub>2</sub>O emissions and potentially increase available Ca concentrations to buffer the soil. We measured soil Ca, pH, NO<sub>3</sub><sup>-</sup>-N, and NH<sub>4</sub><sup>+</sup>-N as well as greenhouse emissions from each replicate aggregate set.

## MATERIAL AND METHODS

The A, E and B horizons of a spodosol soil under a mixed hardwood/softwood forest were used to construct mesocosm to observe the calcium-earthworm-plant interaction for *L. rubellus* (L), *A. agrestis* (A) and a control treatment with no earthworms (N) (Fig. 1). 2 g of soil aggregates (earthworm casting) were taken from these mesocosms of the plant-soil experiment and transferred to headspace vials (Fig. 2). We had two factors: earthworm species and addition/no addition of ammonium, signified as (+) and (-). Treatments are listed in Table 1. The samples were divided into 3 sets. One set for the initial analysis, the second set after 7 days and the third set after 14 days. Ammonium-N was added to half the soils in sets 2 and 3 to achieve 200 µg NH<sub>4</sub><sup>+</sup>-N l<sup>-1</sup> content. For greenhouse gas analyses, the vials were sealed tightly with a rubber septum and incubated for 16 hours. After measurement of CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> with Shimadzu GC, soils were subjected to other measurements. For pH and Ca measurements 7.5 ml distilled water were added and left for 2 hours and then measured with micro-electrode connected pH meter instrument. To measure extractable inorganic N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) after extraction with KCl 2.5 ml 2 M KCl was added to soils and measured colorimetrically with Lachate instrument.

## OBJECTIVES

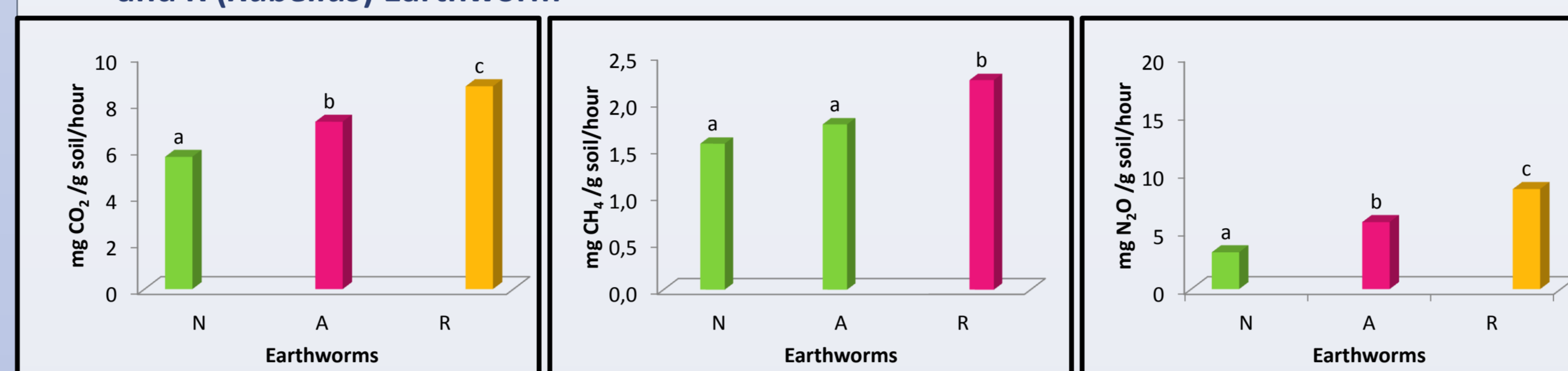
Objectives of this study was to see;

- whether nitrification affects pH,
- whether Ca is involved in buffering acidity,
- whether addition of NH<sub>4</sub><sup>+</sup>-N accelerates acidification and at which rate,
- how will the greenhouse gas emission be,
- to reach to a consideration of microbial activity from release of CO<sub>2</sub> and
- to see the effect of NH<sub>4</sub><sup>+</sup>-N addition on N<sub>2</sub>O release in relation with the earthworm species.

## RESULTS

At the beginning of the experiment *Amyntas* castings showed significant difference from others with higher NH<sub>4</sub> content and lower Ca and pH levels (p<0.05 ANOVA). Initially, NO<sub>3</sub> contents were similar among treatments (p<0.05 ANOVA). But at the two later dates, NO<sub>3</sub> concentrations were greater for earthworm treatments for (-) ammonium treatments. When NH<sub>4</sub> was added, *L. rubellus* treatments had greater NO<sub>3</sub> concentrations. CO<sub>2</sub> and N<sub>2</sub>O results showed significant difference among each other with the sequence *Rubellus* (R) > *Amyntas* (A) > control (N) while only R had a significant higher methane release (p<0.05 ANOVA)(Figure 3).

Figure 3. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O Emission Prior to Incubation for N (Control), A (*Amyntas*) and R (*Rubellus*) Earthworm



At the end of first week of incubation earthworms had significantly greater CO<sub>2</sub> and methane release at (-) sample set (p<0.05) (Figure 4). Ammonium addition was significantly different on methane release (Figure 4). *L. rubellus* with ammonium addition caused significantly greater N<sub>2</sub>O emissions. At the end of second week of incubation, earthworms castings had significantly greater CO<sub>2</sub> release (p<0.05) (Fig. 5) for (+) and (-) applications.

Figure 4. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O Emission After 7 d Incubation for N (Control), A (*Amyntas*) and R (*Rubellus*) Earthworm and Ammonium Addition (+) (-) Applications

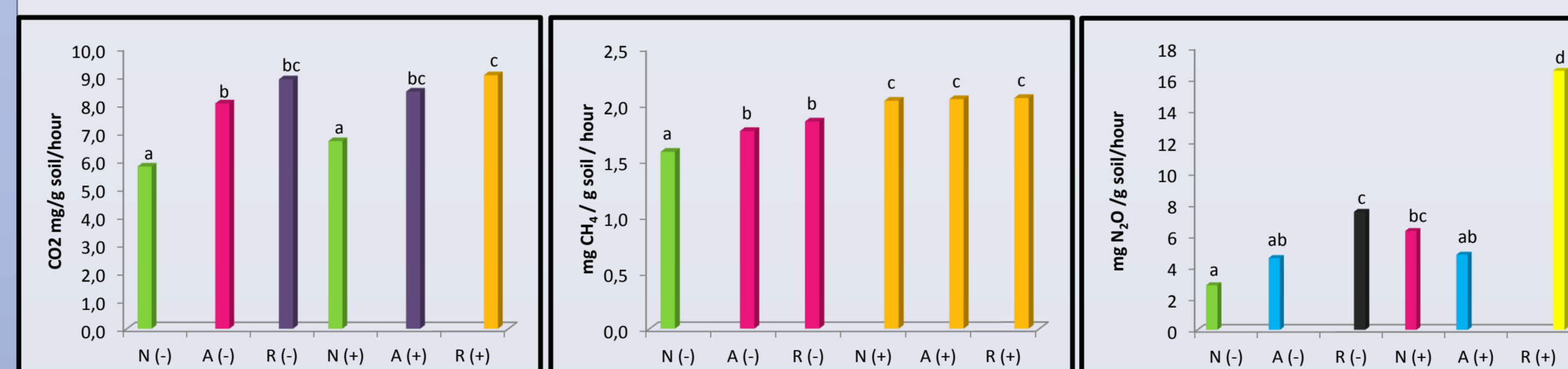
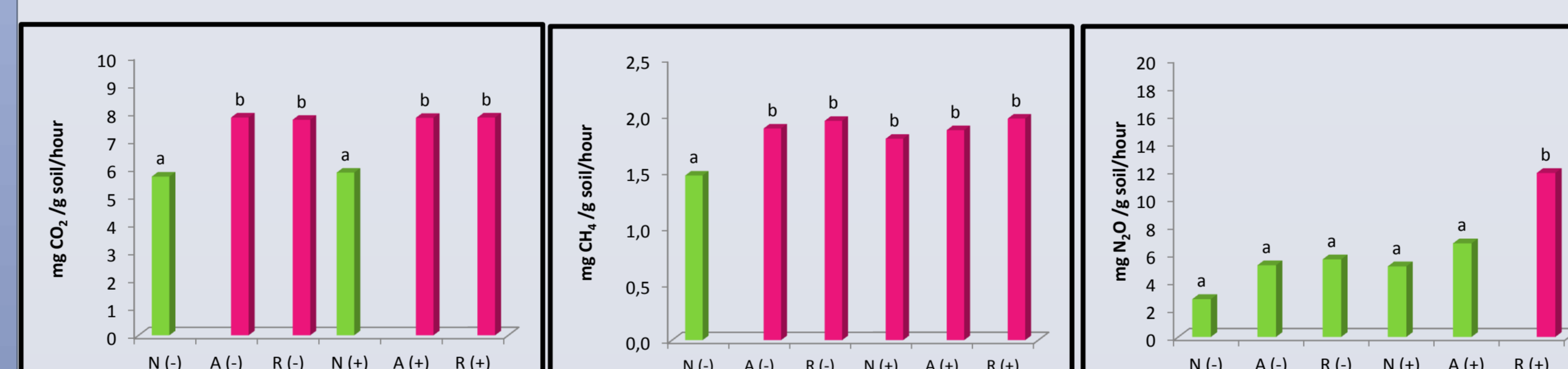


Figure 5. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O Emission After 14 d Incubation for N (Control), A (*Amyntas*) and R (*Rubellus*) Earthworm and Ammonium Addition (+) (-) Applications



Lower pH values in aggregates in earthworm soils than in the N(+) treatment (p(F) < 0.001) may have been caused by nitrification as NO<sub>3</sub><sup>-</sup>-N concentrations in the earthworm treatments were greater than in the N(-) treatment (p(F) < 0.032). Greater water soluble Ca concentrations in the earthworm treatments could not buffer the pH differences. N<sub>2</sub>O and CO<sub>2</sub> emissions were consistently greater for the earthworm treatments in both NH<sub>4</sub><sup>+</sup>-N treatments with the exception of A(+) which emitted significantly less N<sub>2</sub>O than either the L(+) or N(+). For CO<sub>2</sub> in soils without NH<sub>4</sub><sup>+</sup>-N additions, worm type and pH contributed significantly to CO<sub>2</sub> predictions (p<0.0001). For treatments with NH<sub>4</sub><sup>+</sup>-N additions, model predictions (p<0.0096) were mainly influenced NH<sub>4</sub><sup>+</sup>-N concentrations. For N<sub>2</sub>O without NH<sub>4</sub><sup>+</sup>-N additions (p< 0.0001), earthworm type was the only significant contributor to predictions. For N<sub>2</sub>O emissions with NH<sub>4</sub><sup>+</sup>-N additions (p<0.0001), NH<sub>4</sub><sup>+</sup>-N concentrations, NO<sub>3</sub><sup>-</sup>-N concentrations and earthworm type were significant contributors to the predictions.

Table 1 : Summary of treatments

Earthworm	Ammonium addition incubation	Measurement Period	Replication (n)	Total
Control (no earthworm)	No addition	Initial	10	30
<i>Amyntas agrestis</i>	No addition		10	
<i>Lumbricus rubellus</i>	No addition		10	
Control (no earthworm)	No addition	After 7 days	10	60
	Added		10	
<i>Amyntas agrestis</i>	No addition		10	
	Added		10	
<i>Lumbricus rubellus</i>	No addition		10	
	Added		10	
Control (no earthworm)	No addition	After two weeks	10	60
	Added		10	
<i>Amyntas agrestis</i>	No addition		10	
	Added		10	
<i>Lumbricus rubellus</i>	No addition		10	
	Added		10	

We reached proportionally higher levels of relations between NH<sub>4</sub> cc and each greenhouse gas contents especially at control and *A. agrestis* treatments but almost no correlation could be detected for *L. rubellus* after NH<sub>4</sub> addition (Figure 6). There also was a significant correlation between Ca and NO<sub>3</sub> concentration (r<sup>2</sup> =0.528, Figure 7). The relations between Ca and greenhouse gas contents were not high (r<sup>2</sup> values between 0.100 and 0.150).

Figure 6: NH<sub>4</sub>-CO<sub>2</sub> relation along incubation period at N, A and R worm data set.

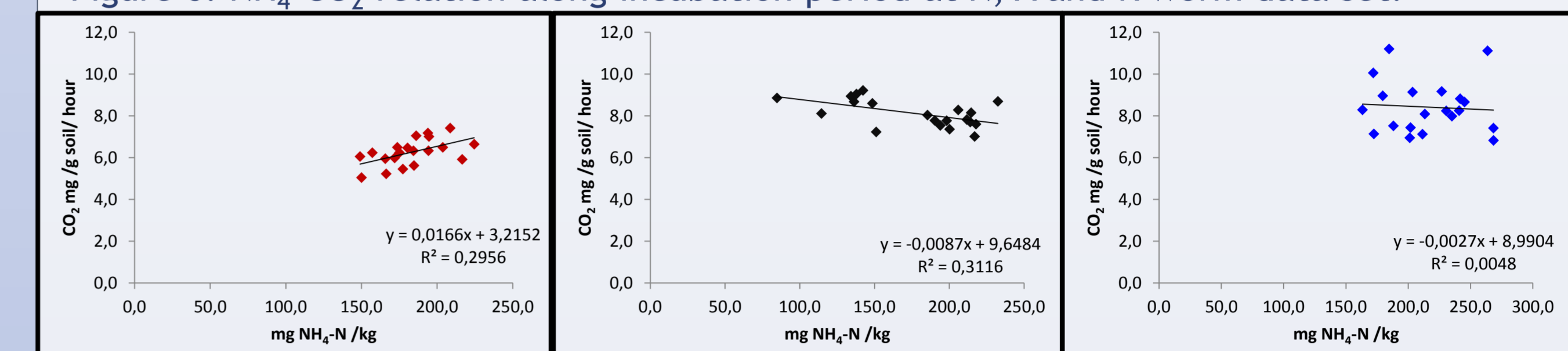
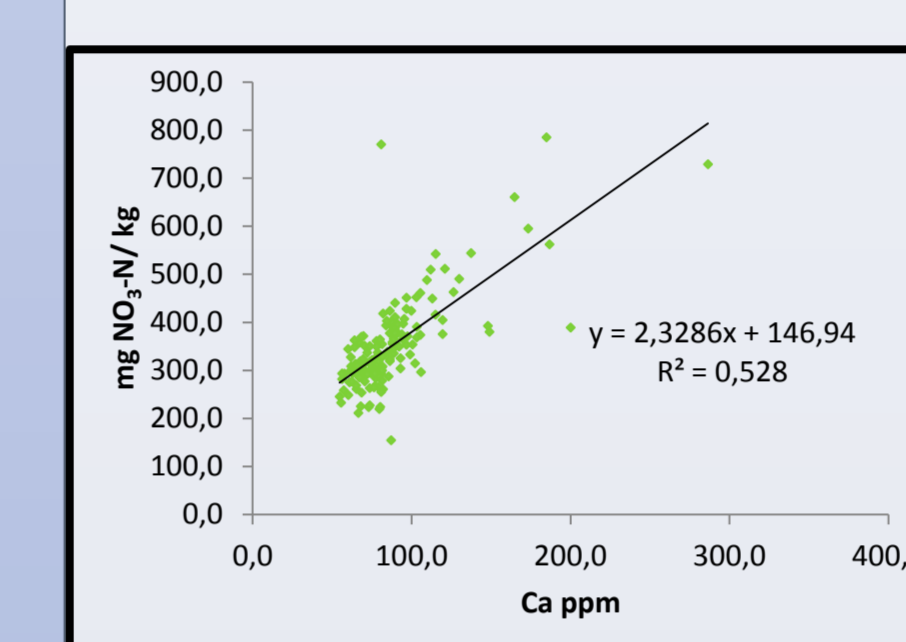


Figure 7: Relation between Ca to NO<sub>3</sub> concentrations.



Pooling all three time points, our results suggest that NH<sub>4</sub> concentration had a significant effect on CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O release especially for the no earthworm control revealing r<sup>2</sup> values 0.297, 0.146 and 0.675 respectively while NO<sub>3</sub> cc did not show good correlation with any greenhouse gas at all worm treatments.

## DISCUSSION & CONCLUSION

Difference of ammonium concentrations among initial values were attributed to the applications along pot experiment. We had expected to see increased pH with ammonium addition as the effect of nitrification was buffered by Ca release (Figure 7). The net effect was that there was lower pH in earthworms soils despite of the increase Ca concentrations. For pH our results were different from some of our field studies and those of others that report an increase in pH when earthworms are present. Our lab study differed in that we looked at aggregate scale effects where microsite effects may override the effect of inter-aggregate soil solution. Our lab study also differed in that the microbial community was separated from the earthworms potentially suggesting that the earthworm needs to “renew” its effect on the community.

Our findings on greenhouse gas emissions resemble those of others (Lubbers et al., 2013). Aggregates taken from earthworm treatments have greater emissions than aggregates from the control soil. Methane is a notable exception after NH<sub>4</sub> was added. Also N<sub>2</sub>O emissions from *L. rubellus* aggregates responded more to NH<sub>4</sub> additions than the two other treatments.

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## CONTACT INFO

H. Barış TECİMEN: baristecimen@gmail.com  
 Josef GORRES: jgorres@uvm.edu  
 Ryan D.S. MELNICHUK: rmelnich@uvm.edu